BIOCHEMISTRY.—*An observation on pufferfish toxin.*[1] Robert D. Macomber, School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, Calif. (Communicated by Bruce W. Halstead.)

Many workers have observed that the skin of pufferfish contains a considerable amount of toxin. Tani (Teikoku Tosho Kabushiki Kaisha **2** (3): 1–103. 1945) reported that some skin samples extracted and assayed contained as much as 20 percent of the total toxicity of the fish. Routine screening tests in our laboratory have also demonstrated puffer skin extracts to be strongly positive.

## MATERIALS AND METHODS

With the foregoing facts in mind, it was suggested that the water used in thawing frozen puffer specimens for preservation be assayed. A mouse was injected intraperitoneally with 1 cc of the water used to thaw a frozen Japanese puffer, *Fugu pardalis* (Temminck and Schlegel). It was found that the water contained sufficient toxin to kill a mouse in about 5 minutes.

After this chance observation, the experiment was set up as follows: From another frozen pufferfish of the same species, samples of flesh, liver, gonad, and skin weighing 7 grams each were assayed to determine the toxicity of various parts of the fish. For the assay, the samples were homogenized in a Waring blender with 2 cc distilled water being added per gram of samples; the skin sample being tough and leathery, was cut in small pieces with the shears prior to homogenizing. Samples were then centrifuged 20 minutes at 2,000 rpm and the supernatant liquid decanted for injection of mice, following the procedure routinely used by Halstead (Copeia (1): 1–11. 1954.)

The skin extract assayed 3.4 mouse units of toxin per gram of sample. (A mouse unit, as recommended by H. Sommer and K. F. Meyer, Arch. Path. **24** (5): 568–570, 1937, in their work on paralytic shellfish poison has been adopted for fish bioassays. The mouse unit is defined as the amount of toxin required to kill a 20-gram mouse in 15 minutes.) From data on the other samples, it was estimated that the skin contained roughly 14 percent of the total toxicity of the fish.

For the second part of the experiment another sample of skin weighing 7 grams was then removed from the fish from an area adjacent to that of the first sample. This second sample was washed thoroughly on the exterior surface with methyl alcohol. A cotton swab wet with the solvent was used to remove all traces of mucus and the skin was scraped lightly with a razor blade. The alcohol wash and scrapings were combined and evaporated to dryness under vacuum. The residue was made up to a volume of 14 cc with distilled water for assay and the residue insoluble in water was not removed. The skin, free from all signs of mucus on the exterior surface, was extracted with water and assayed.

## RESULTS

The skin extract showed toxic symptoms in the mice but they all recovered. The water suspension of the residue from the alcohol wash assayed 3.05 mouse units of toxin per gram of skin sample. The alcohol wash solution, then, contained approximately 90 percent of the amount of toxin that had been present in the homogenized sample of skin extracted with water.

The results are tabulated as follows:

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| Water extract of skin | 3.4 MU/gm of sample |
| Alcohol wash of exterior skin surface | 3.05 MU/gm of sample |
| Water extract of skin sample previously washed with alcohol to remove mucus | Weakly toxic |

## SUMMARY

The major portion of the toxin present in the skin of the Japanese puffer, *Fugu pardalis* (Temminck and Schlegel), appears to be in the superficial layers of the skin or sufficiently near the surface to be removed with solvent wash.

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